Translation into English of a letter to WIPO, dated November 29, 2005

The following observations relating to an inventive step are submitted in response to the corrected International Search Report of October 6, 2005 and the Communication under Rule 43*bis*.1 PCT enclosed therewith:

According to claim 1 of the present application, a method of separating and/or enriching prokaryotic DNA is claimed, said method comprising the steps of:

a. contacting at least one prokaryotic DNA present in solution with a protein which specifically binds prokaryotic DNA and has 25% to 35% homology with the wild type CGPB protein, thereby forming a protein-DNA complex, and
b. separation of said complex.

In contrast thereto, document D1 discloses a method of separating methylated and non-methylated DNA by the use of a protein fragment (MDB) specifically binding methylated CpG islands. This method is a method of fractioning genomic DNA according to different methylation conditions, using a large amount of genomic DNA as starting material (*"100µg"*, D1: p. 234, right column, paragraph 3; i.e. approximately 98 µg methylated DNA and 2 µg non-methylated DNA, cf. D1: p. 236, left column, lines 4 to 6), wherein the protein fragment MDB used for fractioning binds both methylated and non-methylated DNA, although with different strengths.

In contrast thereto, the method of the present invention is a method of separating and/or enriching prokaryotic DNA, based on different proportions of CpG motifs, using a protein which specifically binds prokaryotic DNA and has 25% to 35% homology with the wild type CGPB protein.

In the method according to the invention, three factors are combined for the first time. These factors are based, on the one hand, on the distinctiveness of prokaryotic and eukaryotic DNA. These DNAs differ, on the one hand, in their different amounts of CpG motifs (20 times higher in prokaryotic DNA) and, on the other hand, in their different methylation conditions (methylated in eukaryonts, non-methylated in prokaryonts). Thirdly, the binding specificity of certain proteins/polypeptides with respect to non-methylated DNAs is utilized (cf. p. 7, paragraph 4 of the specification) in order to provide the method according to the invention.

In order to arrive at the purification method of present claim 1 according to the invention, the person skilled in the art would have been required, at the priority date, not only to deduce the enrichment of **non-methylated** DNA from the method of fractioning **methylated** DNA as described in D1, but would also have had to transfer this finding to the enrichment of prokaryotic/viral DNA, **although** the ratio of background DNA (genomic, also genomically methylated and non-methylated!) to target DNA

(pathogen DNA) is considerably inferior than in the above-mentioned case (98:2 methylated versus non-methylated genomic DNA).

Moreover, this is further contrasted by the fact that the requirements for application of the method according to D1 and of the enrichment method according to the invention are completely different. Thus, both the composition of the DNA mixtures (methylated genomic DNA/non-methylated genomic DNA according to D1 versus methylated genomic DNA/prokaryotic non-methylated DNA according to the invention) and the concentration ratios of the DNAs to be separated in each case differ, which further prevented the person skilled in the art from deducting the method of the invention from the method according to D1.

The transfer of said method to the method according to the invention is further prevented by several important features of the method disclosed in D1, which are not compatible with the method according to the invention. On the one hand, the capacity of the column with the bound protein MeCP2 described in D1 would be too low to completely bind the eukaryotic DNA present with a great excess.

On the other hand, the MeCP2 protein used in D1 also binds non-methylated DNA, although said binding is weaker. Further, the volume in which the non-methylated DNA is present after binding of the methylated DNA to the protein does not change with respect to the starting volume. Thus, there is no enrichment in this case.

Inverting this argument, the method disclosed in D1 would thus not be suitable at all to enrich prokaryotic DNA or to separate eukaryotic DNA, respectively. Therefore, the method according to D1 does not suggest the method of the invention at all.

In view of the unfavourable ratio of host DNA to pathogen DNA of $1:10^{-6}$ to $1:10^{-8}$ (cf. the present application, p. 4, second paragraph) and the fact that the protein fragment used in D1 binds both methylated and non-methylated DNA, it was surprising for the person skilled in the art, in particular, that direct enrichment and/or separation of prokaryotic DNA from a solution is possible.

Further, a combination of the teachings of documents D1 and D2 can not suggest the present invention either.

Document D2 describes a transcription-activating factor, namely the human CpG-binding protein (hCGBP). In D2, this factor was shown to play a role in regulating the gene expression in CpG motifs. It was further shown that hCGPB is capable of binding non-methylated CpG motifs. Therefore, the studies in connection with document D2 were carried out in order examine the influence of hCGPB on the transcription of CpG-rich promoters in eukaryonts. However, this document provides no hint that the protein might be capable of recognizing prokaryotic DNA as well. Further, the teaching of document D2 does not give any hint that it is possible using this protein to enrich prokaryotic DNA. As

a consequence, it was not possible for the person skilled in the art to deduct a method enabling separation and/or enrichment of prokaryotic DNA from the teaching of document D2.

The presently claimed method of separating and/or enriching prokaryotic DNA goes far beyond the transcription-regulating function of the protein. The present invention combines the ability of the protein to bind non-methylated CpG motifs with the potential of enriching prokaryotic DNA. As a consequence, a combination of the teachings according to documents D1 and D2 was not suitable to suggest the present invention. Using a combination of binding non-methylated CpG motifs and the potential for enriching prokaryotic DNA, the detection of prokaryotic pathogens with the help of molecular-biology methods is considerably facilitated. Mention should be made, in particular, of the application for diseases caused by pathogens.

In summary, the teachings of documents D1 and D2, either taken alone or in combination, do not suggest the presently claimed invention.

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